Renewable and Sustainable Energy Reviews 15 (2011) 3141–3155

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Monitoring of anaerobic digestion processes: A review perspective

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ARTICLE INFO

Article history:
Received 21 December 2010
Accepted 11 April 2011

Keywords:
Anaerobic digestion (AD)
Process monitoring
Process Analytical Technologies (PAT)
Process sampling
Theory of Sampling (TOS)

ABSTRACT

The versatility of anaerobic digestion (AD) as an effective technology for solving central challenges met in applied biotechnological industry and society has been documented in numerous publications over the past many decades. Reduction of sludge volume generated from wastewater treatment processes, sanitation of industrial organic waste, and benefits from degassing of manure are a few of the most important applications. Especially, renewable energy production, integrated biorefining concepts, and advanced waste handling are delineated as the major market players for AD that likely will expand rapidly in the near future.

The complex, biologically mediated AD events are far from being understood in detail however. Despite decade-long serious academic and industrial research efforts, only a few general rules have been formulated with respect to assessing the state of the process from chemical measurements. Conservative reactor designs have dampened the motivation for employing new technologies, which also constitutes one of the main barriers for successful upgrade of the AD sector with modern process monitoring instrumentation.

Recent advances in Process Analytical Technologies (PAT) allow complex bioconversion processes to be monitored and deciphered using e.g. spectroscopic and electrochemical measurement principles. In combination with chemometric multivariate data analysis these emerging process monitoring modalities carry the potential to bring AD process monitoring and control to a new level of reliability and effectiveness. It is shown, how proper involvement of process sampling understanding, Theory of Sampling (TOS), constitutes a critical success factor.

We survey the more recent trends within the field of AD monitoring and the powerful PAT/TOS/chemometrics application potential is highlighted. The Danish co-digestion concept, which integrates utilisation of agricultural manure, biomass and industrial organic waste, is used as a case study. We present a first foray for the next research and development perspectives and directions for the AD bioconversion sector.

Contents

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1364-0321/$ – see front matter
doi:10.1016/j.rser.2011.04.026

Nomenclature

AD	anaerobic digestion
COD	chemical oxygen demand
CSTR	Continuously Stirred Tank Reactor
FIA	flow injection analysis
FID	flame ionisation detector
FT	Fourier transform
GC	gas chromatography
HPLC	high-pressure liquid chromatography
HS-GC	head space gas chromatography
IR	infrared
LCVFA	long-chained volatile fatty acids
MS	mass spectrometry
NIR	near infrared
OEM	original equipment manufacturer
PLS	partial least squares
PA	partial alkalinity
PAT	process analytical technologies
PCA	principal component analysis
PC	principal component
RMSEP	root-mean-square-error of prediction
SBR	Sequential Batch Reactor
SCVFA	short-chained volatile fatty acids
TA	total alkalinity
TKN	total Kjeldahl nitrogen
TOC	total organic carbon
TOS	Theory of Sampling
UV	ultraviolet
VFA	volatile fatty acids
VS	volatile solids
Σ SCVFA	sum of short-chained volatile fatty acids

1. Introduction

1.1. Major applications of anaerobic digestion

Anaerobic digestion (AD), also referred to as biogasification and biogas production, is a versatile technology platform that can serve many purposes in industry and society. For example, wastewater treatment processes generate vast amounts of sludge, which is expensive to deposit or incinerate. The sludge volume is effectively reduced by means of AD. Moreover, renewable energy in the form of biogas is recovered during the microbial decomposition of the organic part of sludge, significantly reducing the costs for treating wastewater. Another widespread application for AD is dedicated energy production. Recent European political incentives seek to encourage application of AD in agriculture, the main motivators being a strong desire for reducing the environmental impact from agricultural activities and at the same time enhance the utilisation of nutrients applied for crop-cultivation. Finally, also treatment of the organic fraction of municipal solid waste (OFMSW) and organic industrial waste is effectively done by means of AD [1–4].

1.2. Bio-economy

Production of renewable, clean energy can be accomplished in several ways, e.g. by solar energy, wind energy, hydropower and nuclear fusion, which are all documented viable alternatives to fossil fuels. Another route for production of clean, renewable energy is via the biorefinery. A petrochemical refinery is a well-established technology platform that has been providing the industry with essential chemical building blocks for decades, all derived from fossil oil. A biorefinery is the precise biotechnological counterpart, but here the raw materials are cultivated crops, energy crops, and organic industrial residues and wastes. The biorefinery has the advantage over the petrochemical refinery that it is able not only to supply bulk-commodities, but especially also specialised, high-value products such as enzymes, flavour compounds and additives

for the food industry, and important pre-cursors for pharmaceutical production enabling the biorefining concept to be a versatile ingredient and raw material supplier for many industrial and societal sectors [5].

1.3. Resources for bio-refinery concepts

Conventional crops grown for human consumption and for production of animal feed are politically not recommended for energy production. Meanwhile, agricultural residues (sometimes wrongly categorised as wastes) such as straw are currently not being utilised many places and are therefore readily available in abundant amounts. New crops and varieties engineered for the purpose of high yield (and not intended for human consumption) can form the basis of future biorefining concepts. Large unexploited resources are available. These resources include organic waste from the catering and food processing industries, organic by-product fractions from chemical industries, and residues and wastes from agricultural activities. It is not necessarily seamless to integrate these resources into a biorefining concept, as the origin and quality of a specific organic resource or waste can restrict its further use; there are also vast logistical issues in the collection, sorting, storage, and transportation of these potential raw materials, but nothing today's technologies cannot solve easily as soon as the economic and/or political drivers are manifest. Crops grown specifically for biorefining can easily become an integrated part of the interesting sugar platform, which can substitute many of the conventional petro-chemically derived compounds. The basic sugar monomer glucose is the raw material for a large number of proven, industrial biotechnological processes that are capable of delivering a wide range of important industrial chemicals. Hence, crops with high sugar yield, either in the form cellulose fibre bundles or simple sugars, are of great interest for biorefining concepts and alternative crops are available supporting the cultivation conditions in many regions of the world [4–6].

2. Anaerobic digestion in Denmark: a case study

Political and practical hurdles in relation to integration of renewable energy in the form of biogas derived from agricultural resources (manure, energy crops) and industrial organic waste into the public grid are numerous. A country that has overcome many of these barriers and created a somewhat viable system allowing large-scale centralised AD plants to be integrated into society is Denmark. The political as well as the technological development is worth analysing in detail as it can provide valuable input for further dissemination of AD technologies and allow their successful integration into other countries' political schemes.

In the following the background for this successful implementation is described and the remaining barriers for further expansion of the Danish AD sector are discussed.

Agricultural application of AD has led to 60 farm-scale and 22 centralised large-scale biogas plants currently being in operation. The potential of AD in agriculture is far from being exploited. Merely 4% of the available manure is circulated through biogas plants. In addition, vast amounts of agricultural residues and, to some extent, organic industrial waste are still available. The unexploited bioenergy potentials are present in complex lignocellulosic substrates such as wheat straw, which is an abundant resource, as well as easier digestible grasses and silages. This is a common feature of many European countries [4,6,7].

For comprehensive historical details as well as technical description of the prevailing biogas plant concept refer to the key references [8–12].

2.1. Mobilisation of grassroot movements

Immediately following the first energy crisis in 1973, strong grassroot movements pursued the then novel idea of recovering biogas from manure in order to develop an alternative energy source to fossil fuels based on unexploited, natural resources. These endeavours were supported by the Government in office, which initiated several grant and support schemes. The political ambitions reached beyond renewable energy production and also sought to reduce the environmental impact related to agricultural activities (specifically eliminating the risk of sensitive aquatic environments from becoming polluted with excess nutrients applied to farmland). The political will was later translated into visionary and comprehensive strategies for developing and documenting all aspects of biogas technology, economy and effects on society. Furthermore, national energy resources and future potentials from other alternative sources were thoroughly evaluated providing a sound and reliable basis for political decision-making [8,9,12,13].

2.2. Current share of energy production

Domestic energy consumption in Denmark (climate adjusted) reached 864 PJ in 2009, whereof 3.9 PJ originated from biogas production (wastewater treatment plants and agricultural biogas plants pooled). The biogas production has remained constant over the past five years. In 2007, agricultural biogas plants accounted for approximately 60% of the biogas production distributed among 21 large centralised biogas plants and 60 farm-scale plants. Wastewater treatment-related AD processes at 59 municipal sites and 4 industrial facilities contributed with 22% of the biogas production. The remainder share came from methane recovery from landfill sites [14,15].

2.3. The farm-scale biogas plant concept

The original farm-scale biogas plant concepts developed during the 1970s were simple constructions commissioned by local farmers and developed by local black smiths. Lack of knowledge pertaining to optimal adjustment and control of the sensitive biological process often led to diminishing yields, if any. Hence, the economy in these early designs was non-existing. As a consequence, the Ministry of Trade facilitated the creation of a knowledge dissemination group with the purpose of documenting and improving the performance of the emerging Danish biogas sector. Throughout the 1970s and the 1980s many of the original concepts were abandoned due to severe operational problems causing unacceptable downtime [8,12].

2.4. The centralised biogas plant concept

Based on the experience from farm-scale operation, the principles of economy-of-scale soon became a much debated topic. The idea of centralising the AD plant and having manure from livestock production transported back and forth resulted in the first centralised mesophilic AD plant being build in the Northern part of Jutland in 1984. Numerous operational problems questioned whether this design was indeed feasible, but a later reconstruction significantly increased the yield. Furthermore, the new design allowed addition of industrial organic waste to the AD process, which initiated the era of co-digesting manure and industrial organic waste [8,12].

2.5. Main products of centralised AD

Most centralised AD plants in Denmark have an onsite combined heat and power (CHP) generation facility or they export the biogas

to a nearby power plant. Biogas is combusted directly in suitable gas engines or turbines. The produced electricity is sold to the public grid and the generated heat is used either for process heat or sold to the public district heat grid. The latter option requires that the AD plant is located adjacent to infrastructure or industry willing to purchase the heat energy. The economy of a given AD plant is very much dependent on the contracts the board of directors is able to negotiate with energy buyers (municipalities, energy companies, and industry) [8,10,12].

2.6. Veterinarian benefits of AD

From a veterinarian point-of-view, the centralised AD plant offers pathogen and weed reduction. For thermophilic AD plants effective sanitation of the manure is achieved easily. Pasteurisation at 70 °C for duration of 1 h was originally proposed as the benchmark for effective sanitation of manure. This could be accomplished in a separate batch reactor following the main biogas reactor(s). However, a number of alternative operating temperatures and holding times proved to have the same effect on pathogen reduction. For instance, digesting fresh manure at thermophilic temperature (53.5 °C) for a period of 8 h is considered adequate. Hence, practical restrictions exist on the dosage scheme for manure and other raw materials requiring pasteurisation. Farmers supplying manure to the AD plant can be confident that possible deceases from their livestock production are not spread on arable land or transmitted to other livestock farms. Thorough cleaning of vehicles, equipment, and personnel coming into direct contact with raw manure is a necessary pre-requisite and has been implemented at all Danish agricultural AD plants [10,12,16].

2.7. Fertilising benefits of AD

The digestate (co-digested manure and industrial organic waste) has a fairly homogeneous content with respect to major nutrients (N, P, and K), which makes it easier to apply to farmland as fertiliser, since the fertilising value is well-declared. Moreover, essential nitrogen is present on a form (ammonium) that is easily accessible by plants. Using tractor and dragging-hose equipment the digestate is effectively incorporated into the soil without significant loss of fertilising value. Moreover, the digestate is stable organic matter, which acts as a valuable soil enricher [1,10,12,16].

2.8. Socio-economic benefits and externalities of AD

From a socio-economic point-of-view centralised AD plants offer solutions to many challenges encountered in agriculture and rural districts. Digestate has a less offensive odour compared to raw manure. This is important in the context of ensuring good relations between the farmers (crop-cultivators, appliers of manure) and their civilian neighbours in the countryside. Although it is not easy to put a monetary value on this adverse effect, it is critically important when considering possible locations for future biogas plants. Few people are interested in having an offensive smelling AD plant as a neighbour, mostly because of rumours and negative PR associated with ill-operated AD plants. The need for qualified personnel to take care of manure handling, technical service on the AD plant itself and related instrumentation as well as the spin-off activities in local communities is also an important factor. The activity level is generally high and the impact an AD plant has on the local community (service, trade) is significant. On the other hand, the possibility for local or regional communities to become self-sufficient with renewable, clean energy is a strong motivator for many politicians in this day and age. Biogas derived from agricultural resources combined with for instance wind power and solar power can become important key components in the future energy production scheme in rural districts in developed as well as in developing regions [8,10,12,13].

2.9. Issues yet to be resolved

Lack of industrial organic waste of sufficiently high quality has stressed the market severely leading to strong competition between the AD plants themselves and between the agricultural AD sector and the wastewater treatment sector, which is also a keen receiver of suitable wastes for increasing biogas yields. This has initiated many new research projects and programmes trying to identify future raw materials that can be introduced into the AD sector. Pre-treating manure by various low and high technological means is one option, but as stated above, many unexploited biomass resources are still readily available. However, their complex lignocellulosic structure does require advanced pre-treatment steps in order to guarantee a feasible yield from the AD process. Currently, a number of technologies are being investigated in laboratories and at pilot-plant installations, but reliable economic and socio-economic assessments of these new technological possibilities have yet to be disseminated.

Future AD plant concepts will most likely be radically different from the concepts that are prevailing today. This can be seen as a direct consequence of the limited availability of easily digestible quality industrial organic waste and the sharp competition. Also, legislative barriers currently prevent AD plants supplying heat to the public grid from accumulating profit. Recent research efforts in AD seek inspiration in well-known principles from classical chemical engineering disciplines. Serial digestion, where several reactors with varying operating conditions are connected, has shown promising results compared to the conventional design, where only one main reactor is considered.

Although the Danish AD sector has a long and well-documented history, the development in process monitoring and control as seen from a technological point-of-view is not impressive. Many AD plants are relying on a few simple-to-measure parameters, which give a poor indication of the state of the biological process. Unintentional organic overloading, accidental addition of toxic substrates, and other process interruptions occur frequently. Lack of raw material quality control and analysis is believed to be one of the main limitations for effective raw material handling. The performance of an AD plant by and large depends on the qualifications of the process technicians and on the manager [10,17–21]. Here is a tremendous still largely untapped potential for high-skill competence building and for modern process technological involvement, albeit always with a keen eye for capital costs; what is in heavy demand is high-tech low-cost technologies.

3. AD process theory

3.1. General concept

The generally accepted definition of AD is a microbiologically mediated process during which organic carbon present in biopolymers and other degradable compounds is converted to its most reduced form (methane) and its most oxidised form (carbon dioxide) in the absence of oxygen (anaerobic conditions).

It is not the aim of this work to recite the many interesting reactions and phenomena occurring in the AD process. However, for clarity, an overview of the AD process, as seen in many recent textbooks, is given in Fig. 1.

For simplicity, the AD process can be characterised by four sequential processes as depicted in Fig. 1. The characteristic times of the individual AD process steps, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, differ significantly. In a healthy AD

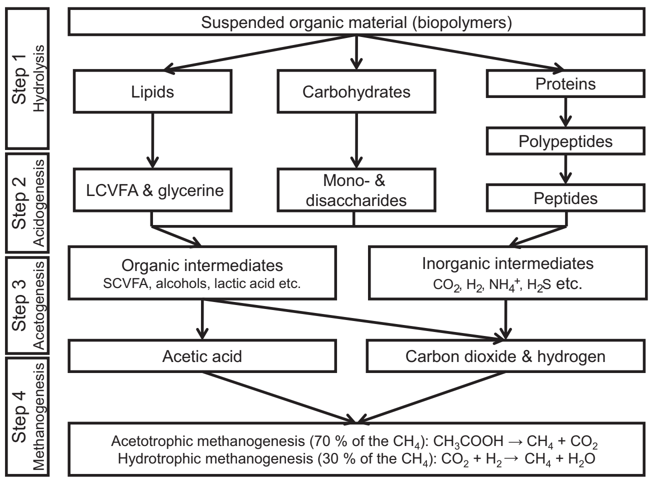


Fig. 1. Breakdown of the anaerobic digestion process. Overview of the four principle reaction steps, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, in the AD process.

process, the duration of the former is usually from days to hours depending on substrate complexity and chemical environment, while the latter proceeds in seconds to minutes. Hence, substrate entering the methanogenesis is converted instantaneously leading to the assumption that volatile fatty acid (VFA) levels should be fairly low in a well-balanced system [22].

3.2. VFA as process indicators

Feasible and relevant indicators of the AD process condition have been argued for decades. Many authors have suggested volatile fatty acids (VFA) as control parameters as these acids are indicative of the activity of the methanogenic consortia. In case of VFA accumulation, this can be interpreted as either organic overload or inhibition of the methanogenic microbial communities due to the influence of other factors. In either case, action should be taken to avoid reactor failure. Relevance of specific VFA acids is still unclear. However, simple short-chained VFA acids (SCVFA) have been the favourites of many researchers [23–27].

A comprehensive survey of the condition of 15 Danish AD plants, both wastewater-related AD and agricultural AD plants, was reported by Karakashev et al. [28]. It was shown that mesophilic plants had greater microbial diversity compared to thermophilic. Furthermore, VFA and ammonium levels were significantly higher for AD plants treating manure than plants treating sludge from wastewater treatment. The 9 agricultural AD plants studied had VFA concentration levels in the range of 3 $g\,L^{-1}$ or less, while ammonium concentrations varied from approximately 2 $g\,L^{-1}$ to 6 $g\,L^{-1}$.

3.3. VFA quantification

Many analytical methods have been developed for quantification of VFA acids of relevance for AD process monitoring. Short-chained VFA acids commonly present in sample matrices such as manure and wastewater sludge are listed in Table 1. Quantification methods vary in complexity and analytical performance. The most important and widespread ones are discussed in the following.

Several authors have suggested different variants of titration methods for efficient and cheap AD process monitoring. This methodology is certainly worth pursuing for AD plants that are not interested in detailed information about the relative abundance of individual VFA acids, but merely seek a measure for total acidity. Methods for use in academia and industry as well as in developing countries have been developed, implemented and validated [29–31].

However, as indicated by the similarities between the pK_a values of the individual VFA acids listed in Table 1, it is not possible to distinguish between the VFA acids using titration methods.

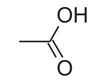
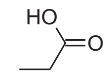
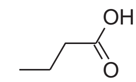
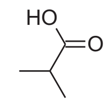
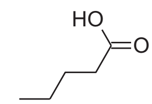
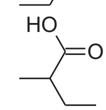
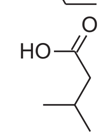
Chromatographic methods commonly found in industry and research laboratories are capable of separating the individual VFA acids and provide quantitative measures of their concentrations. Chromatographic separation is based on relative affinity between a mobile and a stationary phase in a separation column. Again, referring to Table 1, gas chromatographic separation of the VFA acids is feasible due to the relative large differences in boiling points. Brondz [32] presented a comprehensive review outlining the technological and methodological developments for quantification of fatty acids by liquid and gas chromatography. These techniques are routinely applied by professionals and require a great deal of expertise and sample pre-processing. Nevertheless, automated chromatographic systems have been developed and put into work at full-scale AD plants [33–36]. There are some positive experiences from broad-based general process technological applications of chromatographic techniques, some of which may be relevant for the bioconversion field [37,38].

4. Conclusions and recommendations from previous reviews

Mata-Álvarez et al. [1] outlined the technological achievements in handling of the organic fraction of municipal solid waste (OFMSW) using AD. Their work illustrated the complexity of the AD sector, which deals with many different types of substrates and relies on many different processing techniques. The research achievements until year 2000 had mainly focused on process understanding in laboratory- and pilot-scale using well-defined substrates and cultures. Reported studies dealing with pre-processing techniques applied to difficult substrates with the purpose of increasing the digestibility (for instance due to solubilisation of particulate organic matter) were numerous. Also, start-up of AD processes has always been a much debated topic. Many papers have suggested procedures for efficient process inoculation and have been focusing on reduction of the time until the process reaches full production capacity. Significant savings can be accomplished by application of a proper and effective start-up procedure. The authors noted that the industrial interest in AD co-digestion was surprisingly low. Most industrial AD systems were designed to treat a specific type of waste only thereby not benefitting from the advantages of co-digestion systems. The widespread implementation of AD co-digestion plants in Denmark was highlighted as an outstanding development.

Pind et al. [22] presented a comprehensive review on monitoring and control of anaerobic reactors, focusing on general instrumentation and methods for process knowledge acquisition. The review included both low rate and high rate systems. Concerning the much applied CSTR (Continuously Stirred Tank Reactor) systems, the authors made several remarks worth mentioning here as well. Stirred tank reactors commonly have long hydraulic retention times (usually in the order of 15 days or more). The reason for this is lack of biomass immobilisation inside the reactor. To prevent washout of the microbial consortia, reactors are loaded with small amounts of feed periodically. Hence, the reactor configuration resembles a Sequential Batch Reactor (SBR). However, for historical reasons, the term CSTR is used in AD literature. The authors emphasized that the feeding strategy can have significant influence on the chemical environment in the reactor. Process variables (for instance pH, alkalinity, VFA, and gas yield) often display transient responses to the feeding profile. Moreover, the substrates

Table 1
SCVFA, data and structures.

IUPAC nomenclature	Formula	CAS #	MW (Da)	bp (°C)	pK_a	Structure
Ethanoic acid	CH_3COOH	64-19-7	60.1	117.9	4.76	
Propanoic acid	CH_3CH_2COOH	79-09-4	74.1	141.2	4.87	
n-Butanoic acid	$CH_3CH_2CH_2COOH$	107-92-6	88.1	163.8	4.83	
2-Methylpropanoic acid	CH_3CHCH_3COOH	79-31-2	88.1	154.5	4.84	
n-Pentanoic acid	$CH_3(CH_2)_3COOH$	109-52-4	102.1	186.1	4.83	
2-Methylbutanoic acid	$CH_3CH_2CHCH_3COOH$	116-53-0	102.1	177	4.80	
3-Methylbutanoic acid	$CH_3CHCH_3CH_2COOH$	503-74-2	102.1	176.5	4.77	

IUPAC: International Union of Pure and Applied Chemistry, CAS: Chemical Abstracts Service, MW: molecular weight, bp: boiling point, pK_a: dissociation constant. All data from CRC Handbook of Chemistry and Physics, 86th ed., ISBN: 0849304865.

fed to these systems contain relatively large proportions of solids (sand, fibres, and particulate organic matter) compared to other AD reactor systems. Drawing representative samples from the substrate feed line and the reactor is complicated and subject to many sampling errors in general not sufficiently counteracted (or even recognised) due to the highly heterogeneous material involved. Direct sensor interfaces (on-line, in-line, at-line) are subject to microbial growth (fouling), which further complicates data interpretation, as the sensory signals can be corrupted by the presence of biofilm. AD plants are commonly regulated based on at-line or off-line analytical results. Few process parameters are routinely recorded on-line and they are limited to easily accessible data such as pH, redox potential, gas production rate, and flow rates. Human intuition and operator experience rule over dedicated control algorithms. The conclusions were that process parameters preferably should be available on-line and not rely on either human intuition or intervention. Only through application of automated process monitoring and data interpretation could effective control of AD systems be achieved.

Vanrolleghem and Lee [3] reviewed instrumentation for on-line monitoring of wastewater treatment plants. The authors stated that most wastewater treatment plants were designed in such way that the legislative requirements concerning the effluent quality disposed off into recipient water bodies could be guaranteed without the need for comprehensive process monitoring. However, the desire for expansion of the treatment capacity (either larger reactors or shorter retention times) due to general growth in society and industry seeded the idea of intensifying the monitoring effort. Future wastewater treatment plants will presumably be designed to allow greater flexibility, which necessitates comprehensive insight into the core of the AD process in order to avoid unintentional disturbances. This criterion can only be met through careful application of appropriate sensor technology and process modelling. Sensor technologies were divided into two classes; one covering simple, low-maintenance, and reliable sensors, which were appropriate for automatic control systems. The other class comprised advanced sensors. The authors stated that the second class generally requires significant human intervention in the form of maintenance and calibration efforts. Typically, advanced sensors were only applied by specialists (consultants) for audits or in academia for research and not for dedicated on-line process monitoring and control of AD plants.

Spanjers and van Lier [39] analysed implemented instrumentation for AD process monitoring. Wastewater treatment AD plants are associated with high costs. This is mainly due to the conservative design of the reactors, which are very much over-sized to guarantee process robustness. Limited monitoring is included in traditional designs putting the fate of the process in the hands of the plant operator. Introducing reliable monitoring and control technology into the sector would allow AD plants to be operated closer to their effective capacity limit instead of wasting reactor volume due to conservative design rules. The influent (substrate) characteristics fluctuate over time and are partly highly heterogeneous in nature. Wastewater treatment plants are not able to predict future compositions of the received wastewater, because the dynamics of society and industry constantly results in transients. Sudden influxes of concentrated organic material to a wastewater treatment plant can lead to process upsets and severe disturbances, if not counteracted in due time. Equally serious is the presence of toxic compounds (heavy metals, industrial chemicals, and recalcitrant compounds) that pose a severe threat to the biological process itself. Without reliable monitoring systems that respond to such unpredictable transients, the wastewater treatment AD process is as such always at risk. Many of these potential threats can only be detected using proper on-line techniques.

Ward et al. [4] comprehensively reviewed the possibilities for optimising the performance of AD reactors based on agricultural resources. The authors discussed reactor configurations, process parameters as well as feeding strategies in detail. It was noted that many AD processes are operated at suboptimal conditions due to

Spectroscopic	Electro-chemical	Chromatographic	Other
Fluorescence FLU	pH	GC	Acoustic chemometrics a.c.
Infrared IR	Redox potential	GC headspace	Mass spectrometry MS
Near infrared NIR	Electronic tongue ET	HPLC	Microwaves
Raman	Electronic nose EN		Titration
Visual VIS			
Ultraviolet UV			

Fig. 2. Reviewed analytical modalities. Clusterisation of the reviewed modalities into four main classes. The spectroscopic and electro-chemical methods are of special interest, since these two classes are developing significantly at present.

lack of proper on-line data. The complexity of the feed in many agricultural AD plants tends to encourage plant operators to regulate the process conservatively rather than trying to exploit the full biogas potential. Moreover, the monitoring systems currently applied in the AD industry were believed to give a poor and inconsistent view of the actual process state. The use of multivariate sensor technologies and electrochemical arrays was encouraged as many studies have shown promising results in both laboratory-scale and pilot-scale.

5. Review of the modern AD process monitoring potential

5.1. Monitoring of bioprocesses

The technological development within the field of process monitoring in the established fermentation industry is attractive to analyse as it has many similarities with the AD sector. Although the latter has significantly less capital available for instrumentation and labour, state-of-the-art technology development, miniaturisation of components along with economy-of-numbers (mass production) of advanced process sensors are about to cause a change in this paradigm. For a comprehensive overview of the many current interesting advanced sensor alternatives, collectively known as Process Analytical Technologies (PAT), that are available on the market today, we refer to the recent, authoritative text edited Bakeev [38].

5.2. Principles of operation for reviewed monitoring techniques

Focusing on the key metabolites depicted in Fig. 1, the application of numerous monitoring techniques for quantifying these parameters have been reported in the literature. For the sake of providing a clear and comprehensive overview of available techniques, reviewed modalities have been organised in four main groups as depicted in Fig. 2. The general principles of operation constituting the core of these techniques are described in [3]. Below follows a review of reported studies, where these techniques have been brought in to the context of monitoring AD processes.

For in-depth details about the individual measurement principles consult Harris [40]. Vanrolleghem and Lee [3] surveyed implemented and emerging technologies for AD process monitoring, while Bakeev [38] presents a comprehensive work covering multivariate process analysers in all industries and sciences.

5.3. Fluorescence spectroscopy

Peck and Chynoweth [41] reported a study, where fluorescence was monitored on-line along with pH, redox potential and biogas flow in a mixed culture fermentation AD process. VFA acids were quantified off-line using gas chromatography. The fluorescence probe was immersed in the culture and a single wavelength was used for excitation and emission respectively. It was concluded that the fluorescence signal from NAD(P)H, which is directly related to metabolic activity, gave the earliest warning of process imbalance due to organic overload compared to the other measured process variables. The authors suggested fluorescence monitoring as being a promising alternative technique for early indications of process disturbances in AD processes.

Another fluorophore is the coenzyme F_{420}, which is specific for methanogenic bacteria. Although probes are available for quantification of this intracellular compound, the questions of interpretation and the significance of the signal in relation to AD process stability still remain open [3,41].

Morel et al. [42] equipped an AD reactor with a recirculation loop, in which a fibre optic probe was placed. AD substrates contain many fluorophores that can interfere with for instance the fluorescence signal from NADH. Several excitation and emission wavelengths were used in order to overcome reported interference problems. By application of multivariate methods it was demonstrated that fluorescence spectroscopy could predict VFA as well as chemical oxygen demand with reasonable accuracy.

5.4. Infrared spectroscopy

Steyer et al. [43] demonstrated the use of infrared spectroscopy for determination of VFA, COD, TOC, and total and partial alkalinity in an industrial AD reactor. An ultra-filtration unit was used to provide a clear liquid free of particles to be passed on to the instrument. The authors argued that the emerging market for fibre optics soon would allow for in-line IR analysis of AD processes using fibre optics, which would greatly expand the uses of IR in industry. The IR spectrometer was calibrated using samples from a real process based on the argument that interactions between chemical species in complex matrices lead to that the resulting spectrum differing from a simplistic linear combination of the spectra from the individual chemical species involved. Hence, an extensive calibration with synthetic samples is not feasible. The authors followed the AD process for several months and were able

to document process upsets and failures based on analysis of the IR spectra.

Spanjers et al. [44] also followed an industrial AD process for several months with an IR spectrometer equipped with a CaF_2 transmission measurement cell. The instrument was modified and a pre-treatment unit was added allowing particles to settle in the withdrawn sample, before being sent to a 150 μm filter followed by the measurement cell. It was argued that the calibration models were associated with high uncertainties due to low concentrations of the analytes (COD, VFA, bicarbonate, phosphate, nitrate, nitrite, ammonium, and TKN) and hence low absorbances in the IR spectra. It was concluded that the pre-sedimentation and filtration did not perform optimally, as occasionally clogging occurred. The solution was a cheap alternative to commercial ultra-filtration units.

5.5. Near infrared spectroscopy

Nordberg et al. [45] reported the use of near infrared spectroscopy for monitoring of an AD reactor in laboratory scale fed with synthetic substrate. The probe was immersed in the reactor. Disturbances were introduced to the system and it was noted that immediate changes were observed in the near infrared spectra. Data were analysed using chemometric multivariate methods and the authors concluded that the calibration models for VFA (acetate and propionate) were acceptable for the purpose of on-line measurements, although they did not fully match the performance of the reference methods (chromatography).

Hansson et al. [46] applied near infrared spectroscopy for monitoring of an AD laboratory-scale process fed with the organic fraction of municipal solid waste (OFMSW). Several attempts were made to deliberately stress the AD process. It was noted that for minor disturbances in the form of organic overloading, the AD process stabilised itself after 12 h. However, for an aggressive overloading, propionate accumulated leading to a drop in pH of 0.3 pH units. Moreover, the biogas composition significantly changed. Based on multivariate analysis of the data it was concluded that propionate models were better and more robust than acetate models. The reason for this was unclear.

Holm-Nielsen et al. [47] reported an experimental work, where samples from four Austrian agricultural AD reactors were analysed off-line using an innovative flow cell coupled to a standard near infrared spectrometer, the TENIRS system. Primary samples from the full-scale reactors were brought to the laboratory. The flow cell incorporated a maceration step, where particles, straw in particular, could be effectively reduced prior to scanning. Multivariate calibration models were made for the parameters individual VFA acids, ammonium-N, TKN, and VS. It was shown that the measurement principle allowed for adequate modelling of the parameters of interest. The authors indicated that due to the promising model performance the system would be applied immediately on both pilot-scale (150 L) and full-scale (1800 m^3) installations. This study acknowledged primary sampling issues, which were not optimised yet.

Holm-Nielsen et al. [48] investigated the responses from laboratory AD reactors by adding easily digestible glycerol to laboratory AD reactors operated on manure and observing the effects. Three 5 L reactors operated at thermophilic temperature were equipped with re-circulating loops allowing on-line near infrared monitoring and representative sampling of the heterogeneous bioslurry (using the TENIRS system mentioned above). Organic overloading was successfully induced, which completely stopped the biogas production. Rapid accumulation of glycerol and VFA was seen from off-line analysis. The authors concluded that a stable AD process with increased biogas yield could be achieved by adding 3–5 $g L^{-1}$ glycerol. Furthermore, it was shown that by use of multivariate models it was possible to predict both VFA acids (acetate and propionate) as well as glycerol from the near infrared spectra with acceptable accuracy and precision.

Sarraguça et al. [49] monitored an activated sludge reactor with near infrared spectroscopy. An immersion probe with a 10 mm path length was mounted in the reactor. Spectra were acquired daily and the probe was withdrawn from the reactor and cleaned thoroughly to avoid interference from fouling. Multivariate methods were applied for data analysis along with several variable selection techniques. The authors noted that the limited range of the parameters investigated (COD and nitrate) combined with the strong absorbance of water in the near infrared spectral region, probably were the main reasons for the poor correlations obtained in the study. This study suffered from an experimental design evergreen problem: lack of proper analyte span; this is an error very often seen in both laboratory as well as full-scale studies.

Zhang et al. [50,51] used near infrared spectroscopy for characterisation of the effluent from an anaerobic hydrogen-producing reactor fed with synthetic wastewater. Multivariate calibration models were made for ethanol, acetate, propionate, and butyrate. The modelling performance was optimised through application of various types of spectral pre-processing methods. Meanwhile, the relatively high number of reported PLS-components applied for the multivariate regression models indicate that there is a risk of over-fitted data.

Lomborg et al. [52] surveyed the performance of near infrared spectroscopy for monitoring of agricultural AD reactors in laboratory scale co-digesting manure and maize silage. It was argued that energy crops such as different types of silages already are being integrated in AD processes worldwide and that there is an immediate need for proper on-line monitoring of such installations. The TENIRS flow cell system earlier described by Holm-Nielsen et al. [47,48] was also applied for these experiments. The authors concluded that near infrared spectroscopy in conjunction with multivariate data analysis is capable of providing real-time information about the AD process. Specifically, multivariate calibration models were made allowing prediction of all the SCVFA acids of interest as well as dry matter.

Jacobi et al. [53] reported the use of near infrared spectroscopy for monitoring of a full-scale agricultural AD plant processing maize silage. It was stated that according to German authorities, 4000 agricultural AD plants were in operation in Germany by the end of year 2008. The majority of these plants were not equipped with instrumentation allowing the operators to fully optimise the AD process. In the reported study, a specially designed measurement head was implemented at the AD plant. A campaign of 500 days duration formed the basis for the results. Multivariate calibration models were made for the two VFA acids acetate and propionate. It was concluded that the applied routine of referencing the instrument once a week probably gave rise to errors in the spectral data. Technical improvements, including automatic referencing, will be the theme for further work.

5.6. Ultraviolet and visual spectroscopy

Redondo et al. [54] designed a flow injection analysis (FIA) system, which allowed quantification of sulphide in fermentation broth and hydrogen sulphide in the gas phase. Such measurements are relevant in relation to biogas cleansing as well as AD processes in general. The system consisted of one detection module and two sample-modules, which allowed quantification of said species on the same analyser. The results indicated excellent performance of the system with low detection limits suitable for AD process monitoring.

5.7. Electronic tongues and noses

Rudnitskaya and Legin [55] reviewed the possibilities for monitoring biotechnological processes using electronic tongues and noses. The authors emphasized that recent applications of chemical sensors coupled in arrays have overcome many of the earlier reported barriers for their successful implementation. The most important of these hurdles is low selectivity. By proper use of multivariate methods, the sensor arrays can be optimised and outperform many techniques found in a typical analytical laboratory. The low price and robustness of these sensors make them viable alternatives to modern complex analytical hardware for monitoring of complex processes. However, common problems such as fouling, degradation due to the harsh chemical environment and sampling issues are also themes that have to be dealt with for this type of sensors. Meanwhile, gas phase measurements by use of chemical sensors has a great potential, as many of the problems encountered in the broth are absent in the gas phase.

Nordberg et al. [45] implemented chemical sensors (Metal Oxide Semiconductors) in the headspace of an AD reactor. By use of multivariate methods, the sensor data were correlated with the concentration of volatile compounds. Good accuracy was obtained for both methane and SCVFA (acetate and propionate), whereas hydrogen gave suboptimal results. The authors argued that the technology was still maturing and that technological development would severely decrease costs and strengthen the potential in the future to come.

Buczkowska et al. [56] reported the construction of a minituarised array of chemical sensors for monitoring of a laboratory-scale AD process. The sensors were arranged in a modular set-up and could be integrated in a recirculation line. Applying multivariate methods, acceptable calibration models were constructed for VFA, COD, and pH. It was argued that changes in process conditions, in particular pH, apparently had an influence on model performance. Dividing the data set into two classes based on their pH value (high and low, respectively) gave improved modelling statistics for acetate. A patent application was filed for the modular design of the flow cell.

5.8. Gas chromatography

Pind et al. [33] presented a novel measurement principle based on in situ membrane filtration. A gas chromatograph was connected to the system allowing automatic VFA quantification with a frequency of 15 min in the range from 0 to 3000 mg L^{-1} of all the SCVFA acids. The authors argued that no filtration system had previously been validated in the literature. By optimising the parameters for the filtration, the authors showed that the filtration unit could be operated for a period of 100 days without the need for replacing components in full-scale. An equivalent of 200 samples could be analysed automatically without any intervention from technicians. This work resulted in a patent application.

Boe et al. [34] developed a method based on headspace gas chromatography. A special sample pre-treatment cell was designed. By adjusting temperature, pH and increasing the ionic strength in the sample, the solubility of volatile compounds was lowered drastically. The gas phase was sampled, analysed by gas chromatography, and the concentrations of individual SCVFA were determined using solubility relations. The authors stated that the method had a superior advantage, since it did not involve the use of any filtration units prone to fouling and clogging.

Diamantis et al. [35] described a method based on capillary gas chromatography suitable for process monitoring and research. An automatic sampling module was applied followed by a filtration unit. Injections into the gas chromatograph were performed automatically. The authors showed that the system responded to and verified sequences of organic overloading of the reactor.

Boe et al. [36] further refined the principle they described earlier for use on a laboratory-scale AD system. Again, the main driver for using headspace gas chromatography was the effective elimination of fouling problems associated with filtration units. A rugged system was designed based on previous experience and known complications, when analysing biomass from agricultural AD plants. For a period of 6 months, the system delivered on-line determinations of SCVFA from a laboratory-scale AD reactor treating cow manure subjected to various feed pulses in the attempt to affect the SCVFA levels. It was discussed that effective mixing of the reaction cell had to be considered to eliminate problems caused by mainly foam formation due to addition of acid. Small sample volumes were preferred for several reasons in this design. However, the authors argued that future prototypes would take into account the benefits of larger sample volumes, larger loop sizes, etc. in the effort of optimising the response from the gas chromatograph.

5.9. Titration

Feitkenhauer et al. [29] presented an economical alternative for SCVFA quantification based on titration. The titration system was tested on a laboratory-scale AD process treating synthetic textile wastewater. Their solution facilitated analysis of samples without the need for filtration. A specially designed measurement cell was employed in which titration was performed. The system was controlled by a computer, which automatically adjusted the titration procedure based on the expected SCVFA concentrations. The robustness of the method was tested by adding salt simulating varying wastewater quality from industrial processes. The method was not significantly affected by these changing salt levels. Based on the performance and the simplicity of the method it was concluded that it could be readily applied for analysis of any AD process.

Jantsch and Mattiasson [57] described a titrimetic method for monitoring of partial and total alkalinity in AD processes. The measurement system was based on the flow injection analysis (FIA) principle and was completely automated. A laboratory-scale AD reactor was equipped with a recirculation line connected to the FIA system through a filter, which allowed analysis of a sample every 8 min. The reactor was monitored for a period of several months. As a reference to the FIA method, titration was applied. The results of the regression analyses showed excellent agreement between the two methods. The authors noted that the system needed plenty of attendance, which must be optimised, before the concept can be applied in an automated process environment.

Lahav and Morgan [30] reviewed simple titration methods for AD process monitoring with a special focus on developing countries. One of the opening remarks in the paper was that titration is superior to any other routine monitoring method with respect to simplicity and cost. This still holds true, maybe also at many AD plants in developed countries. The many proposed titration methodologies published over the years were discussed critically. The authors noted that these methods, although simple in nature, are capable of providing accurate results concerning the state of an AD process. Operators are free to choose from a number of alternative methods, ranging from simple two-point titrations to eight-point titrations involving advanced calculations. In conclusion, much dependent on the AD reactor and system behavior, a suitable titration technique can always be used for on-site evaluation of the process state, although the resolution and detection limits are inferior to expensive analytical hardware.

Molina et al. [31] reported the installation of an automated titrimetic system, AnaSense, for pilot-scale monitoring of an indus-

trial AD process treating wastewater. The system was based on two-point titration. It was argued that multi-point titration methods were less applicable at industrial scale. The pilot-scale reactor was fed with synthetic wastewater. Throughout the measurement campaign, on-line determinations of total dissolved organic carbon were provided by a TOC-analyser. These values were used to calibrate the titration system under the assumption that dissolved carbon was due to SCVFA. Periodically, the agreement between the AnaSense and a gas chromatograph was checked. The authors concluded that the system was capable of providing an estimate with resolution of between 5 min and 10 min. The performance of the system at low SCVFA concentrations gave unstable predictions, however, a clear trend could be observed in the data, which is sufficient for process monitoring and control.

5.10. Microwaves

Nacke et al. [58] reported a system for quantification of dry matter in bioslurry based on microwaves. The measurement range was from 2% to 14% dry matter. The principle of operation was based on the pronounced difference between dielectric constants of water (approximately 80) and solids (approximately 2–10). The microwave radiation could be sent through plastic pipes or other plastic process instrumentation without significant damping of the signal. A laboratory-scale reactor was inoculated with digestate from an agricultural biogas plant. The dry matter content in the reactor was varied from 3% to 10%. Uni-variate and multivariate models were built and showed good performance. However, the authors argued that for real-world applications a multivariate approach should be considered as the dielectric variables possible could be influenced by other constituents in the bioslurry rendering uni-variate models useless. It was pointed out that the microwave sensor was not prone to suffer from common problems encountered for spectroscopic techniques such as fouling and clogging of the cell. The principle was truly non-invasive.

5.11. Acoustic chemometrics

Lomborg et al. [52] investigated the feasibility of using passive acoustic measurements and chemometrics to model the dynamics of dry matter concentration changes during AD in laboratory-scale. Three 5 L reactors digesting manure were fed with maize silage in the effort to increase the dry matter level. The TENIRS unit described above was modified to also include an accelerometer integrated into the loop (non-invasive). The dry matter was increased from 4.8% to 11.4% resulting in 14 different levels. Multivariate calibration provided models with acceptable accuracy and precision for prediction of the dry matter content. This study is a role model for the type of “remote sensing” acoustic chemometrics application potential available today; see also [59,60] and Section 6 (Table 2).

6. Discussion

6.1. Quantification of relevant process parameters

Whether an AD process is designed to treat high-strength industrial wastewater, sludge from municipal wastewater treatment plants or co-digest agricultural resources and organic residues a number of relevant process parameters and monitoring issues are in common. Classical process parameters include simple-to-measure parameters such as pH, temperature, and redox potential. These parameters are all relatively easy to quantify on-line using commercially available technology. For at-line or off-line determinations, there is no need for extensive training of the personnel performing the analysis. However, these parameters provide only very limited insight into the complex biochemical network of coupled reactions that occur in an AD process.

Comprehensive insight into the dynamics of the microbiological process can be obtained, if the monitoring program is augmented to also include volatile fatty acids (VFA), ammonia/ammonium and off-gas composition. Conventional determination of these parameters requires technically more complex analytical hardware and highly skilled personnel. Recent advances in development of (multivariate) process sensors have made it possible to substitute these maintenance-heavy methods with rugged and reliable process sensors, the sensor-analyte quantification being based on multivariate calibration [61]. There is still a need for specialist intervention, as most (multivariate) process analysers require extensive calibration and validation, chemometrics. However, once validated the process analysers provide the plant operator with detailed information about the state of the process.

The present authors suggest the monitoring principle outlined in Fig. 3 as the optimal solution for detailed deciphering of process dynamics in practically any chemical or biochemical reactor.

6.2. Emerging PAT modality: Raman spectroscopy

To the knowledge of the authors, no studies concerning Raman spectroscopic monitoring of AD processes have been reported so far.

Raman spectroscopy is widely recognised as being complementary to infrared spectroscopy. Moreover, the signal from the dominant compound in AD processes, water, is much less pronounced in Raman spectra than in for instance near infrared spectra. Most importantly, the superior selectivity for compounds present in trace amounts in complex matrices has been proven in numerous studies. From the literature [38,62,63] it becomes clear that the technological development within the field of Raman spectroscopy has resulted in several interesting solutions over the past few years. It is possible that Raman spectroscopy will be one of the key methods for bioprocess monitoring in the near(er) future, as many of the reported hurdles (for instance interference from fluorophores) can be overcome by proper application of laser excitation wavelengths and data processing.

6.3. Sensor interfacing issues

An AD process is a harsh chemical environment, which may easily compromise process sensors. Many studies report the use of ultra-filtration or similar measures as a necessary prerequisite to obtain a homogeneous, particle-free liquid that can be analysed. Electrochemical sensors (ion-selective electrodes, electronic tongues, and electronic noses) can degrade due to poisoning of the cell membrane and are subject to growth of biofilm (fouling). Similarly, optical interfaces for spectroscopic techniques require attention as these can be subjected to fouling too and erosion due to fine particles (for instance sand) in the bioslurry flowing by the optical window.

6.4. Sampling issues

A very common assumption in chemical engineering is to assume homogenous mixtures allowing for easier process modelling; it is easy to appreciate, why this assumption is popular. In reality however, biotechnological processes such as AD are far from constituting homogenous systems. The problem of acquiring representative sensor signals and representative process samples for sensor calibration and validation is well recognised in the literature, but mostly just as lip service. Many studies and technology reports continue to describe, report and implement procedures that do not qualify as representative. Since most of the parameters of interest

Table 2
Reported modelling performance of modern modalities for AD monitoring.

Parameter	Range	Modality	Scale	Mode	Reference
Σ SCVFA	40–105 mmol L^{-1}	Titration	Lab	ES	Feitkenhauer et al. [29]
Σ SCVFA	13–4900 mg L^{-1}	Titration	Pilot	ES	Molina et al. (2008)
Σ SCVFA	"Classification"	ET	Lab	IS	Buczkowska et al. [56]
Σ SCVFA	200–800 mg L^{-1}	IR	Full	IS	Spanjers et al. (2008)
Σ SCVFA	0.31–10.20 g L^{-1}	NIR	Full	IS	Jacobi et al. [53]
$H_2S_{(g)}$, $S^{2-}{}_{(aq)}$	0–10,000 ppm	FIA	Lab	ES	Redondo et al. [54]
HAc	0–18 mmol L^{-1}	GC	Pilot	ES	Diamantes et al. [35]
HAc	5–50 mmol L^{-1}	Titration	Lab	ES	Salonen et al. (2009)
HAc	250–600 mg L^{-1}	IR	Full	IS	Spanjers et al. (2008)
HAc	500–1700 mg L^{-1}	IR	Lab	ES	Steyer et al. [43]
HAc	0.07–3.08 g L^{-1}	NIR	Full	IS	Jacobi et al. [53]
HAc	0.14–1.72 g L^{-1}	NIR	Lab	IS	Nordberg et al. [45]
HAc	1–13 g L^{-1}	NIR	Lab	IS	Lomborg et al. [52]
HAc	0–6 g L^{-1}	NIR	Lab	IS	Zhang et al. [50]
HPr	0–18 mmol L^{-1}	GC	Pilot	ES	Diamantis et al. [35]
HPr	0.01–7.47 g L^{-1}	NIR	Full	IS	Jacobi et al. [53]
HPr	0.2–2.8 g L^{-1}	NIR	Lab	IS	Hansson et al. [46]
HPr	0.06–1.78 g L^{-1}	NIR	Lab	IS	Nordberg et al. [45]
HPr	0–6.2 g L^{-1}	NIR	Lab	IS	Lomborg et al. [52]
HPr	0–800 mg L^{-1}	NIR	Lab	IS	Zhang et al. [50]
ind. SCVFA	0.1–50 mmol L^{-1}	GC + filtration	Lab/full	ES	Pind et al. [22]
ind. SCVFA	150, 40, 20, 10 mmol L^{-1}	HS-GC-FID	Lab	ES	Boe et al. [36]
PA, TA	2–3 g $CaCO_3$ L^{-1}	FIA, titration	Lab	ES	Jantsch and Mattiasson [57]
TS	1–10 wt%	Microwave	Lab	IS	Nacke et al. [58]

ind.: individual, lab: laboratory-scale, pilot: pilot-scale, full: full-scale, IS: in situ, ES: ex-situ, Σ SCVFA: sum of short-chained volatile fatty acids.

are dissolved in the liquid phase, filtration is often applied to get rid of the heterogeneous, interfering material such as particulate organic matter, straw, and sand particles, which unfortunately is not always the case, and therefore cannot serve the role as a universal feature that circumvents the need for documentable, reliable representative samples and/or sensor signals. It is necessary to face this issue squarely in the future and to incorporate already from design phase the principles promulgated by the Theory of Sampling (TOS).

Coppella [64] as well as Holm-Nielsen et al. [65] discussed the benefits and drawbacks of recycling lines and methods for assuring representative samples from complex geometries such as reactors. The principle of operation for most AD reactors involves the use of mixed cultures. Often, for agricultural systems, through regular supply of fresh manure it is assured that microbes that are washed out are being replaced with fresh biomass securing microbial diversity and stability. The high solid content for AD reactors fed with manure (usually in excess of 50 g L^{-1}) plus the presence of straw and other particles, complicates spectroscopic (optical) measurements. Moreover, formation of gas bubbles in the broth is the nightmare of spectroscopists. Usually these issues are counteracted in industry using so-called de-bubblers and macerators, where appropriate. A clear, particle-free and gas bubble-free liquid is much preferred in conventional spectroscopy.

However, the forced technical simplicity of agricultural AD plants must be taken into account. Sophisticated hardware solu-

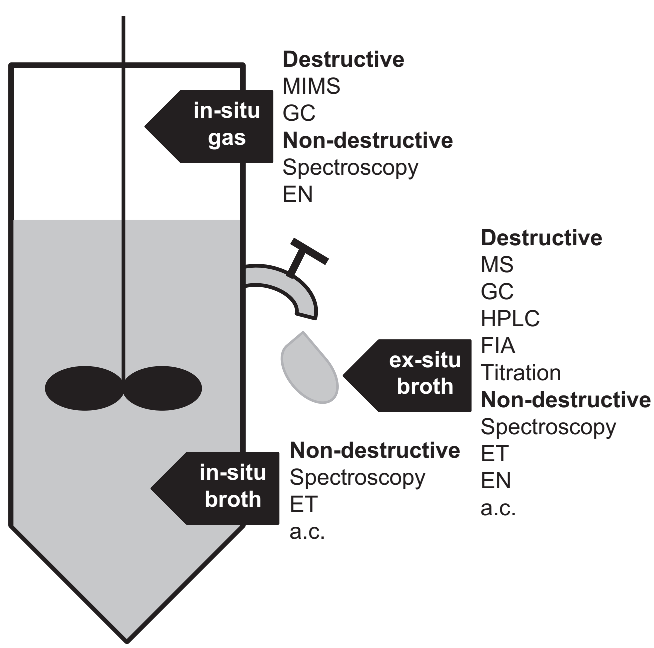


Fig. 3. Analytical modalities put into context. A general PAT monitoring scheme applicable for any chemical, environmental or biochemical process. The in situ interfaces to both the liquid and the gas phase can be accomplished with commercial hardware. Adding a battery of (destructive) reference modalities for at-line analysis further increases the knowledge level pertaining to virtually any process.

tions have to be avoided as documented by the many failed attempts to introduce even only moderately advanced systems in practical, full-scale agricultural AD plants. From a spectroscopic point-of-view this implies that transmission cells prone to clogging due to presence of particles, complicated auto-calibration systems and other convenient measures probably not are applicable. Instead the reflection principle (180° backscatter) should be used, wherever feasible.

Holm-Nielsen [60] presented a first foray of a new holistic view on monitoring the complex AD process in particular and of bioconversion processes in general. This study focused on (but did not solve to completion) the critical role of always being able to acquire both representative samples as well as sensor signals, before the power of chemometric calibrations is applied. A next step in this direction was taken by Esbensen and Julius [66], who laid out the full power of representative sampling, chemometrics as well as using proper validation approaches (test set validation), that are critically necessary in order to prove reliable process monitoring systems. Proper representative sampling is a critical success factor *sine qua non* for all of the issues included in the present review. The reader is referred to Esbensen and Paasch-Mortensen [67], chapter 3 in Bakeev [38] for in-depth treatment of all relevant process sampling issues whether pertaining to sampling systems or to sensor systems.

6.5. Uni-variate versus multivariate data analysis

Relatively few studies on monitoring and control of AD processes handle data using multivariate methods. Instead, classical uni-variate techniques are put into play and used extensively for analysing responses from sensors. Matrix effects (chemical interferences, etc.) are hardly detectable using these techniques. Uni-variate methods are inadequate, when it comes to extracting relevant information from often large, complex data sets. Provided that the specific information is not apparent from the data structure (for instance linear correlation between absorbance and concentration of analyte as determined at a single wavelength) uni-variate methods will never give rise to a robust and valid model.

Many established and emerging sensor techniques output multivariate responses meaning that a single sensor or array delivers many response variables for instance spectra or a series of voltages. The selectivity of one of these response variables with respect to the concentration of a single analyte is often poor. However, the pattern hidden in the response variables provide a sort of fingerprint, which holds vast amounts of meaningful information provided that appropriate data analytical methods are applied to extract this hidden information.

Multivariate data analysis has its root in analytical and physical chemistry. Hence, this discipline was named chemometrics. It is however important to underline that the functionality, power, and versatility of chemometric methods allow them to be applied on data originating from almost any process, not only related to chemistry and a more proper term for this discipline would be multivariate data analysis.

The widespread implementation of spectroscopic techniques has accelerated the development of capable data handling and interpretation algorithms. Frequently, full spectra obtained from one or more process analysers are delivered to the data interpreter. It is obvious that such large datasets not only contain information relevant for quantification of one or more analytes, but also provides vast amounts of auxiliary information, which is not relevant for the problem at hand. Multivariate methods, such as principal component analysis (PCA), allows the original data structure (**X**, the spectra) to be decomposed into two matrices; one that carries relevant spectral information pertaining to the problem and a residual matrix ("errors"). By performing this linear algebraic manoeuvre, the effective dimension of the dataset can be reduced significantly, since only information relevant for describing the analyte variation in the spectra is kept in the data matrix. For a case, where near infrared spectra have been acquired composing of say 1000 different wavelengths (equalling a 1000-dimensional variable space), the PCA will typically be able to reduce this to a dimension of less than 10 principal components. Since the principal components are constructed in such way that they do not correlate (they are orthogonal), it is possible to interpret them independently. Using proper plots of calculated score and loading values, it is possible to detect clusters/classes of samples, which are similar based on their spectral information. Also, abnormal samples (outliers) reveal themselves easily during PCA analysis. Such use of PCA analysis is often applied in data analysis and has been reported in many studies pertaining to AD process monitoring.

The next step, to make actual regression models capable of predicting for instance SCVFA, COD, and dry matter simultaneously from a single spectrum, involves use of multivariate regression methods such as partial least squares regression (PLS regression). As for any modelling exercise, proper validation must always be applied, when implementing multivariate models. Careful planning of experimental details often helps creating the necessary variation in the calibration data. However, AD processes are sensitive systems of microorganisms and variation can be difficult to induce on demand, if the process is stable. The widespread use of internal validation (cross-validation, re-sampling) is by many authors considered adequate. Unfortunately, this kind of validation usually provides over-optimistic modelling performance. The true performance of a prediction model can only be estimated by use of proper external validation (independent test set). The reader is referred to Esbensen and Geladi [68] for an in-depth critique of the serious misuse and mal-practice of cross-validation within applied science and technology.

For details on PCA and PLS refer to Wold et al. [69]. Höskuldsson [70] and Geladi and Kowalski [71] describe the theory behind the workhorse in multivariate data analysis, partial least squares regression. Esbensen [72] gives an introduction to these basic chemometric methods based on geometrical projection understandings.

6.6. Acoustic chemometrics

Among the emerging PAT modalities that have shown promising results for non-invasive process analysis, but which has not at all seen enough exploration, acoustic chemometrics range high. Numerous innovative applications have been documented over the last 15 years showing that this technique is capable of addressing monitoring problems in science and industry that are not easily assessable with other demonstrated modalities, both because of its desirable non-invasive features ("clamp-on" sensor) and because of its complementary nature, mainly addressing physical parameters e.g. average particle size, particle size distribution, bubble concentration and others. The rationale behind the success of this approach is that practically all processes and unit operations emit an acoustic pattern, which reflects the state, and very often also reveals information about composition and phases, of the system/process. It has been documented that even minute changes in hydrodynamic behavior, for example pipe line flow, are qualitatively detectable and very often also quantifiable, using proper reference methods to calibrate the system. Acoustic chemometrics rests squarely on the foundation of multivariate calibration; chemometric data analysis is a critical success factor for extraction of relevant features in the complex acoustic pattern picked up by the applied sensors. Applications for acoustic chemometrics

in AD process monitoring could include monitoring of dry matter dynamics, hydrolysis rate determination for particulate matter, and assessment of wear and tear of machinery and process instrumentation. Halstensen and Esbensen [73] give recent introduction to acoustic chemometrics (and refer to the background technical literature); an easily accessible foundation for acoustic chemometrics is [59].

6.7. Handheld and mobile process analysers—part of the new paradigm?

Recent advances in the field of mobile spectroscopy have resulted in several innovative products. Infrared, near infrared, and Raman analysers are now available in battery-operated mobile and even handheld configurations. These units provide a whole new range of possibilities for process monitoring, largely still unexploited, but the central issue is very clear: instead of bringing the samples to the instrument/laboratory—why not bring the analytical instrument to the process? And why not use a mobile, preferably handheld, analytical instrument, instead of implementing a fixed, dedicated PAT system for full versatility and flexibility? These devices are specifically designed with user-friendliness in mind; hence, they can be operated by personnel having received only a minimum of training. However, this desirable feature does not eliminate the need for competent supervision. First forays certainly appear sufficiently positive to warrant continued application studies [74,75]. Moreover, the structural similarities between these instruments and their laboratory/bench top analogues allow for calibration models to be transferred seamlessly from one type to another [76].

It is therefore possible that the future of process monitoring for AD plants lacking sufficient capital for upgrading to state-of-the-art in-line/on-line analysers to some extent can be substituted by such handheld, mobile instruments, e.g. shared between several AD reactors, AD plants, and/or farmers. The potential application scenarios can only be glimpsed at this stage: exciting studies and results will abound.

7. Conclusions

As documented by this review, there is not a clear winner for AD process monitoring. Robustness, simplicity, accuracy, precision, and reliability are some of the key parameters any AD plant operator has to consider, when focusing on purchase of any of the offered technologies at the market. Many small, local or regional agricultural AD plants can probably survive using simple titration methods and occasionally having their process verified by some sort of consultant service. This could hold for AD plants digesting a mono-substrate (for instance maize silage), where operation experience is well-documented.

Larger, centralised, more complex AD plants, the Danish concept or similar, where co-digestion of manure and various sorts of industrial organic wastes happens at thermophilic temperatures, requires a more reliable and comprehensive approach. Simple measures for acidity and other key parameters will probably not reveal the true process state for all relevant combinations of the feedstocks. Moreover, the lack of quality control of incoming raw materials is a serious drawback. Raw material control should be an integrated part of the process analysis hardware package.

The present authors perceive that the future of anaerobic digestion process monitoring is facing a paradigm shift. Conventional AD reactor design for wastewater treatment plants, where a sufficiently large volume efficiently acts as a buffer and guarantees effluent quality, is no longer attractive. By proper application of modern sensor technology and multivariate data analysis the process can be kept within specifications even at significantly higher loads than seen today.

For agricultural AD plants, robustness and simplicity must necessarily always be part of the process analyser design. The users (farmers) will never adapt sophisticated systems that require expert audit and frequent consultancy by technicians—but as soon as derivative generations of such systems have been suitable simplified, and scaled down economically without compromising the potential, revolutionary information quality and quantity delineated in this review, the market for such robust and technically simple systems is enormous, e.g. as evidenced by more than 5000 agricultural AD plants currently in operation in Germany alone. The degree of robustness and affordability that can be achieved today puts e.g. rural Chinese or Indian markets within reach as well—there is no limit for the commercial OEM sector.

The possibility for expanding the use of closely related specially designed manure analysers to also allow on-field quantification of nutrients applied to farmland during fertilising seasons presents another huge unexploited market worldwide. Many interesting ongoing projects are looking into the possibilities for this kind of deployment.

8. Future research and development

It is recommended that the AD community – together with representatives from industry, agriculture, OEM, and academia – seek to formulate a common strategy for research and development efforts based on the modern approaches for achieving advanced monitoring and control. A perusal of public research databases reveals that it is far from the rule that current well-funded projects are based on the state-of-the-art levels described above, which inevitably leads to redundant research being performed as well as suboptimal utilisation of grants and schemes for applied research. There is a vast, still only barely tapped potential in involving the modern, proven approaches of PAT and TOS for more reliable AD process monitoring and control.

It is hoped that based on the vast experiences relating to effective and advanced process monitoring and control gained from the established fermentation industry mainly rooted in pharmacy and food science, the AD community will be able to benefit by identifying easily transferable know-how. Cost-of-ownership for modern robust, inexpensive process analysers has decreased to a level, where also minor companies (and soon individual farms) can benefit from their implementation. Although it presumably is difficult to change the culture and mentality of all current AD plant operators, immediate proof-of-concept studies can be designed, planned, and executed within the framework of already established research programmes. Conservative AD plant designs are no longer viable options in the modern economy. Capable modern technological means must be adapted in order to assure the best possible process operation conditions and hence maximise the efficiency of the AD sector as a whole.

Acknowledgements

Michael Madsen acknowledges Aalborg University for funding through Ph.D. scholarship no. 562/06-7-28027.

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